ORIGINAL ARTICLE

Phylogeography of a marine acanthocephalan: lack of cryptic diversity in a cosmopolitan parasite of mole crabs

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ABSTRACT

Aim Little is known about phylogeography and cryptic diversity of parasites in the marine environment. The acanthocephalan *Profilicollis altmani* parasitizes intermediate hosts that are broadly distributed around the Americas and final hosts that are highly motile. We investigated the spatial genetic structure of this acanthocephalan found in three species of *Emerita* crabs: (1) to test whether land masses serve as biogeographic barriers promoting ocean basin divergence among parasite lineages or species; and (2) to test whether the distribution of parasite species matches the distribution of different crab host species.

Location The Pacific, Atlantic and Gulf coasts of the USA, and the Pacific coast of Panama and Chile.

Methods Sequences of cytochrome *c* oxidase subunit I (*COI*) and ribosomal internal transcribed spacers (ITS) were obtained from 204 acanthocephalans. Parasites were sampled from crabs in 15 sampling localities. These sequences were analysed with coalescent-based methods and other population genetic analyses to infer phylogeographic patterns.

Results Haplotype diversity for *COI* sequences was high (0.96) among parasites sampled, but nucleotide diversity was low (0.071) and there was no distinct geographic pattern between regions. Pairwise genetic distances were generally low, although there was a degree of population structure between oceans. Sequence comparisons showing an excess of low divergence alleles and a bimodal mismatch distribution provide evidence of either past selective events or demographic expansions. No variation was observed in the ITS sequences.

Main conclusions The lack of geographic patterning in haplotype diversity of this parasite indicates that gene flow is probably occurring between ocean basins. In addition, the low genetic diversity suggests that the acanthocephalan parasitizing *E. analoga* in Chile is conspecific to the species found parasitizing several *Emerita* species along the coasts of North America, and is thus a cosmopolitan parasite that is most likely dispersed long distances by marine birds that serve as definitive hosts.

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INTRODUCTION

Phylogeographic studies that examine genetic diversity have dramatically increased in recent years, and have included studies on numerous marine fish, invertebrates and marine plankton (Bernardi *et al.*, 2003; Baus *et al.*, 2005; Richlen *et al.*, 2008). The biodiversity of marine taxa and their geographic ranges are continually being revised as studies continue to uncover cryptic species, i.e. species that were not previously recognized based on morphology, but that are

genetically distinct (Knowlton, 2000). Among the high diversity of marine organisms is additional unseen biodiversity, as nearly all marine organisms are parasitized by multiple types and species of parasites (Marcogliese, 2007). In contrast to the number of studies on free-living species, few studies have examined the genetic diversity of marine parasites, particularly in a spatial context (Aiken *et al.*, 2007; Plaisance *et al.*, 2008), although knowledge of the diversity and distribution of parasites is essential to understanding how parasites may impact aquaculture and the functioning of marine and estuarine ecosystems (Horwitz & Wilcox, 2005; Rohde, 2005; Hudson *et al.*, 2006).

Cryptic species of marine invertebrates have been described from taxa with widely varying dispersal capabilities and distribution (Knowlton, 2000; Janosik & Halanych, 2010). Like their host organisms, marine parasites often have life stages with varying dispersal capabilities. The encysted eggs and larvae of some parasites may travel with currents in the same way as the planktonic larvae of many marine species; however, parasites can also be transported by the movement of their hosts, allowing parasites to overcome barriers to dispersal that limit other marine species (Whipps & Kent, 2006). Acanthocephalans, or thorny-headed worms, are a group of endoparasitic worms that are more closely related to Rotifera than to other parasitic worms (Garey *et al.*, 1996; Welch, 2000). We examine the phylogeography of a marine acanthocephalan parasite, common in intertidal mole crabs, some seabirds and sea otters, in order to test for the presence of suspected multiple species (Royal *et al.*, 2004) and to determine whether geographically partitioned host crab species support divergent parasite lineages.

Mole crabs (*Emerita* spp.) are an ideal host with which to test hypotheses on marine acanthocephalan parasite divergence patterns because they are highly abundant in sandy beaches and are found over a wide geographic range in multiple ocean basins. Observational and experimental studies suggest that many acanthocephalans use a broad range of definitive host species but are specific to only a few intermediate hosts (Kennedy, 2006, pp. 52–74). The mole crab acanthocephalan parasite appears to conform to this pattern; crabs of the genus *Emerita* are the only known intermediate hosts while a variety of shorebirds and diving birds (hereafter birds) serve as definitive hosts. Thus, sampling the intermediate hosts allows us to better characterize parasite spatial patterns of genetic diversity.

Six species of *Emerita* crabs inhabit sandy beaches around the Americas, and at least three of these are known to harbour species of acanthocephalans from the genus *Profilicollis* – formerly *Polymorphus* (Nickol *et al.*, 2002; Delgado, 2005). Molecular methods have provided an especially useful tool for examining the biodiversity of parasites, which are often difficult to delimit based on limited morphological characters and morphological plasticity (Amin & Redlin, 1980). Three species of acanthocephalan were originally described from *Emerita* crabs and the birds that consume them: *Profilicollis altmani* (Perry, 1942), *Profilicollis*

kenti (Van Cleave, 1947) and *Polymorphus texensis* Webster, 1948, although after further analysis it was proposed that the three species are synonymous and should be referred to as *Profilicollis altmani* (Karl, 1967; Nickol *et al.*, 2002). However, the consolidation of these species into *P. altmani* has not been widely adopted (Royal *et al.*, 2004; Smith, 2007), and another acanthocephalan, *Polymorphus bullocki* (Mateo, Córdova and Guzmán, 1982) is also proposed as a distinct morphological species infecting the mole crabs of Chile and Peru (Balboa *et al.*, 2009). We present new molecular data to help resolve the taxonomy of this parasite by testing whether the acanthocephalans infecting mole crabs in North and South America are genetically distinct, as well as whether there are morphologically cryptic species found in mole crabs.

Of the approximately 1000 species of acanthocephalan described, intraspecific and cryptic genetic diversity have been examined in three freshwater and brackish species: *Pomphorhynchus laevis*, *Leptorhynchoides thecatus* and *Neoechinorhynchus golvani* (Perrot-Minnot, 2003; O'Mahony *et al.*, 2004; Steinauer *et al.*, 2007; Martínez-Aquino *et al.*, 2009). Steinauer *et al.* (2007) investigated *L. thecatus* in multiple fish definitive host species and found that high variation in host use could be explained by genetic divergence among at least six cryptic species, while Martínez-Aquino *et al.* (2009) and Perrot-Minnot (2003) found that the genetic divergence observed in both *N. golvani* and *P. laevis* suggested the presence of two cryptic species sharing an amphipod intermediate host species. Similar patterns of cryptic diversity within intermediate hosts have also been uncovered in several marine digenean trematode parasites (Miura *et al.*, 2005; Leung *et al.*, 2009).

The identification of multiple cryptic species in these aquatic parasites with life cycles similar to that of *Profilicollis altmani* led us to hypothesize that we would observe multiple evolutionary lineages or cryptic species in this acanthocephalan, as suggested in some previous reports. The degree of mobility of a parasite's hosts is expected to be the main contributor to population structure and gene flow within many parasite species (McCoy *et al.*, 2003; Criscione & Blouin, 2004; Louhi *et al.*, 2010); therefore we also hypothesized that migration of birds along each coastline would lead to minimal population structure within each coastline, while significant genetic differentiation would be observed between ocean basins. The phylogeography of the *Emerita* crabs has been examined (Tam *et al.*, 1996) and analysis has suggested that populations of *E. analoga* in the Northern Hemisphere have been isolated from those in the Southern Hemisphere for approximately 1.5 million years (Dawson *et al.*, 2011) while the genetic diversity of their acanthocephalan parasites is unknown. Here, we provide the first biogeographic study of genetic diversity in a marine acanthocephalan, testing for the presence of cryptic species, as well as differentiation between coasts of North and South America to elucidate patterns of dispersal of this parasite.

MATERIALS AND METHODS

Sampling

Five species of *Emerita* crabs were sampled for acanthocephalan parasites: *Emerita analoga* was collected from ten sites along the Pacific coast, *Emerita talpoida* was collected from four sites along the Atlantic and Gulf coast, *Emerita rathbunae* was collected from along the Pacific coast of Panama, *Emerita benedicti* samples were collected in Texas, and *Emerita portoricensis* sampled from Puerto Rico (Fig. 1, and see Appendix S1 in Supporting Information). No acanthocephalans were found in samples from Texas and Puerto Rico.

Mole crabs collected in California and along the east coast of the USA were frozen prior to dissection, while crabs from other sites were preserved in ethanol. A total of 783 acanthocephalan parasites were recovered from the 658 crabs dissected in this study. Parasites from frozen specimens were soaked in deionized water to allow them to excyst from the cystacanth stage to aid later DNA extractions. All parasites were rinsed with deionized water to remove crab tissue and then stored in 95% ethanol.

Genetic techniques

To examine cryptic diversity and population structure in this acanthocephalan, two loci were chosen: mitochondrial cyto- chrome *c* oxidase subunit I (*COI*) and the ribosomal internal transcribed spacers ITS1 and ITS2. These loci were chosen because of the utility of mitochondrial genes for phylogeography (Avise, 2009) and because both *COI* and ITS were previously used to identify cryptic acanthocephalan species (Steinauer *et al.*, 2007). Extractions were performed on approximately 15 acanthocephalans per location. A total of 204 acanthocephalans were sequenced at the *COI* locus (Appendix S2). Of the 15 parasites sequenced for the *COI* gene per population, five with divergent haplotypes were sequenced at the ITS regions, but no ITS variability was observed among the 90 samples.

DNA was extracted from parasites using a modified proto- col from the NucleoSpin Tissue Kit (Machery-Nagel Inc., Bethlehem, PA, USA) and precipitated using 7.5 M ammo- nium acetate and isopropanol. A 604 base pair sequence of the *COI* gene was amplified using modified Folmer *et al.* (1994) primers and a thermocycling protocol from García- Varela & Nadler (2006) and the ITS and 5.8S regions were amplified following Král'ová-Hromadová *et al.* (2003). Tem- plate DNA was amplified in a 25 μL PCR reaction mixture containing 1.5 mM MgCl$_2$, 1 × PE buffer, 0.2 mM dNTPs, 0.5 μM of forward and reverse primers, and 1.0 unit of Taq Polymerase (NEB, Ipswich, MA, USA). Gene fragments were visualized in 1% agarose gels and cleaned with a shrimp alkaline phosphatase-exonuclease enzyme reaction (USB Corp., Cleveland, OH, USA), and cycle sequenced with Big
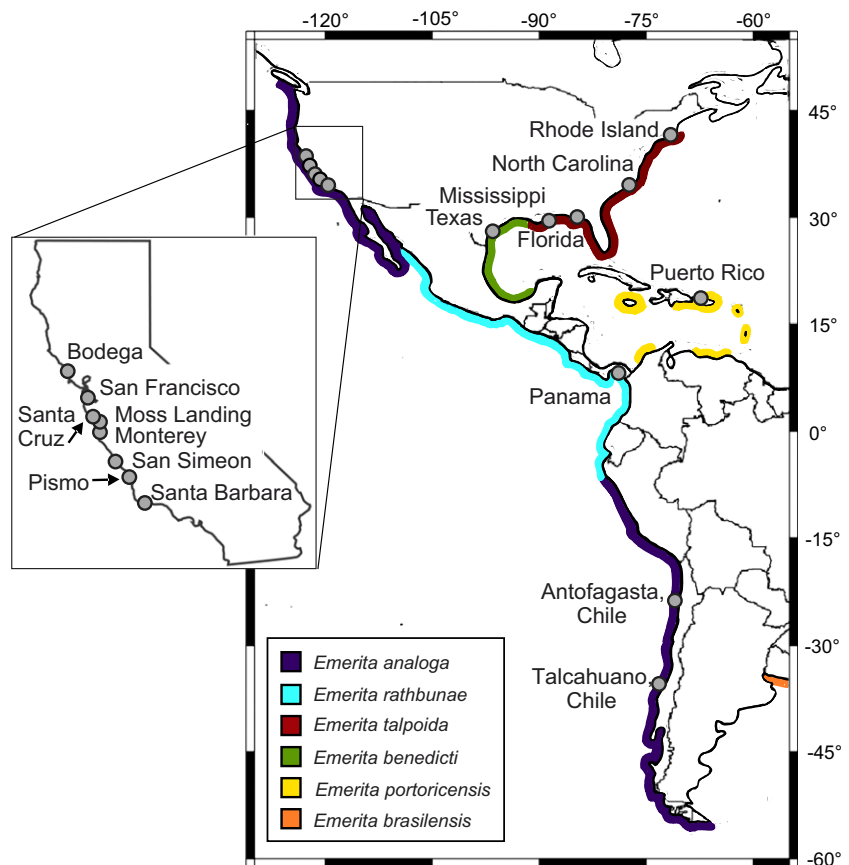

Figure 1 Sampling sites. Shaded circles indicate mole crab (*Emerita* spp.) collection sites; inset shows Californian sites. Mole crab species (*Emerita* spp.) distribution map modified from Tam *et al.* (1996).

Dye v.3 (Applied Biosystems Inc., Carlsbad, CA, USA). Samples were sequenced with an ABI 3130 Genetic Analyzer at the Romberg Tiburon Center genetics lab. *COI* sequences were deposited in GenBank under the accession numbers KF835281–KF835351. The ITS haplotype matches previous ITS sequence AY532066.

Host species identification of *Emerita* crabs was determined for samples from the Gulf coast of Florida and Mississippi by sequencing the *COI* gene to compare to identified specimens in GenBank (Tam *et al.*, 1996).

Population analysis

COI and ITS sequences were aligned separately in Sequencher 4.8 (Gene Codes Corp., Ann Arbor, MI, USA). Standard diversity indices including haplotype diversity (h), mean number of nucleotide differences (π_1) and nucleotide diversity (π_2) were estimated for each population using Arlequin 3.1 (Excoffier *et al.*, 2005). To estimate whether non-neutral evolutionary forces such as selection or past changes in population size had a significant effect on the *COI* locus, Fu's F_S statistic was calculated in Arlequin, and Tajima's D and Fu & Li's D^* statistics were estimated using DnaSP 5.10 (Tajima, 1989; Fu & Li, 1993; Fu, 1997; Rozas *et al.*, 2003) and were considered significant when the *P*-values were less than 0.05. Synonymous and non-synonymous mutations were analysed in mega 4 using the invertebrate mitochondrial genetic code (Tamura *et al.*, 2007).

Sequences were grouped into 14 population sequence sets for analysis in DnaSP based on sample location. Fixation indices (F_{ST} and Φ_{ST}) between populations were estimated in Arlequin, Φ_{ST} incorporating the Tamura and Nei model as the closest model available to the GTR+G model chosen in model comparisons (see below). Based on these values, populations were grouped into two regions (Pacific: Chile and California; Atlantic: western Atlantic and Gulf of Mexico) for an analysis of molecular variance (AMOVA), again using the Tamura and Nei model. Mismatch analyses were conducted with Arlequin with 500 bootstrap replications to compare the frequency of pairwise distances to the sudden expansion model. A Mantel test of isolation by distance was performed using the program Isolation by Distance 1.52 (Bohonak, 2002). A statistical parsimony haplotype network was constructed using all haplotypes in tcs 1.2.1 (Clement *et al.*, 2000).

To compare migration rates between the Pacific and Atlantic Ocean basins to those between California and Chile, an isolation-with-migration model was implemented in IMa (Hey & Neilsen, 2007). The HKY model (Hasegawa *et al.*, 1985) was applied to allow for multiple substitutions. Because there is no specific estimate for mitochondrial mutation rates of an acanthocephalan, a mutation rate averaged from across several invertebrate groups equivalent to 0.021 substitutions per site per million years (from Lynch *et al.*, 2006) was used in calculating an approximate range for the time in years since divergence of the populations.

Preliminary analysis in IMa indicated that simple linear mixing with five chains and the default heating parameter was sufficient to maintain adequate levels of mixing. To verify convergence, the analysis was run three times for each comparison with different random seeds and it was confirmed that the parameter estimates were similar. A nested model was implemented in IMa to test different models of migration between populations. Theta values from the IMa analyses (Appendix S3) showed that for the Pacific to Atlantic comparison there were seven appropriate models to consider and for the California to Chile comparison there were 10 models to test (Appendix S3). A Bonferroni correction was applied to correct for multiple comparisons across these nested models (Carstens *et al.*, 2009).

Phylogenetic analysis

The total number of *COI* haplotypes was calculated using DnaSP 5.10 and the unique haplotypes were used in phylogenetic analyses. The acanthocephalan *Polymorphus minutus* was used as an outgroup (GenBank: EF467865.1). Phylogenetic analyses were conducted using both maximum parsimony and Bayesian criteria. mega 4 was used for maximum parsimony analysis, with random addition trees to begin the search and 500 bootstrap replications. A likelihood approach, implemented in MrModelTest 2.3 was used to determine the mutational model that best fit the data (Nylander, 2004). The GTR+G model was the best fit model by both Akaike information criterion (AIC) values and hierarchical likelihood ratio tests (hLRT), and was used for the Bayesian analysis implemented in MrBayes 3.1 (Huelsenbeck & Ronquist, 2001). Chains were run for 4,000,000 generations with trees sampled every 100 generations until the average deviation of split frequencies was 0.009. The first 10,000 trees were eliminated as burn-in and the remainder used to construct a majority-rule consensus tree. The clades observed in the Bayesian tree were collapsed if posterior probabilities were less than 60.

RESULTS

Phylogenetic and population analysis

The 604 bp fragment of the *COI* gene from 204 acanthocephalan individuals representing 14 localities was characterized by 71 haplotypes, with 43 of these occurring in only one individual (Appendix S2). It was characterized by 71 polymorphic sites, 31 of which were parsimony-informative; 14 of the mutations were non-synonymous and 57 were synonymous. Non-synonymous mutations were rare with each occurring in only one individual. The *COI* locus was used to reconstruct a phylogeny due to the absence of variable sites observed in the ITS loci. The Bayesian phylogeny (Fig. 2) shows two clades, but neither of the clades appears to reflect geographic specificity.

Nucleotide diversity (π_2) was low among all locations and ranged from 0.0067 to 0.0104 (Table 1). Haplotype diversity
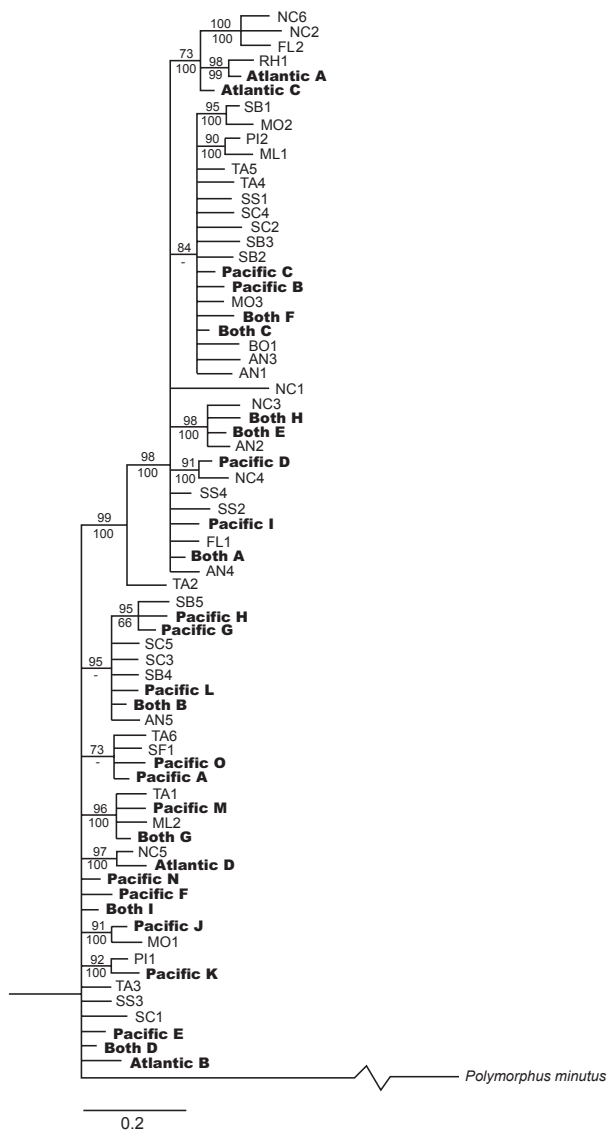
Figure 2 Phylogenetic relationships among *Profilicollis altmani* estimated with Bayesian analysis of *COI* sequence data. Numbers above branches show Bayesian posterior probabilities (> 60), numbers below show bootstrap support values for maximum parsimony analysis (− indicates bootstrap values < 60). Bold haplotypes indicate shared haplotypes from Pacific populations, Atlantic populations, or haplotypes shared between coasts. Singleton haplotypes are indicated by location abbreviations (found in Table 2).

(h) was high between 0.90 and 1.0, and the average pairwise distance between haplotypes was five (Table 1). The haplotype network revealed that rather than one central common haplotype in the network, there were eight common haplotypes, each with several closely related haplotypes found in only a few individuals (Fig. 3). Most haplotypes are separated by one or two base changes, although the network is split into two groupings which show divergence of approximately five to six nucleotides. Many of the common haplotypes were shared between Pacific individuals and Atlantic

individuals and there was also no distinct geographic pattern in the haplotype network.

Pairwise comparisons (F_{ST} and Φ_{ST}) of *COI* sequences from sampled locations showed an absence of population structure between most localities, despite a direct geographic distance between them as large as 8800 km; Φ_{ST} values between all populations ranged from −0.052 to 0.284 (Table 2). Pairwise distances were very low between populations in California; most values ranged between −0.052 and 0.047, although a pairwise distance of 0.113 was calculated between the San Francisco coast and Bodega populations, but was not statistically significant ($P = 0.063$). Comparisons of pairwise distances between California and Chile showed similarly low pairwise distances of −0.032 to 0.029. Few of the pairwise distances were statistically significant, and the majority of the significant comparisons (81%) were between Atlantic and Pacific localities. These pairwise distances varied from −0.025 to 0.226, and were mostly significant between Florida and the Pacific populations. Interestingly, only one comparison of Rhode Island to Pacific populations was significant, while comparisons of pairwise distances between Rhode Island and the two other Atlantic/Gulf populations were also statistically significant and similar to distances between Atlantic and Pacific localities.

An AMOVA analysis suggested that genetic diversity is indeed different between Pacific and Atlantic regions ($\Phi_{CT} = 0.081$, $P < 0.001$), although this accounts for just 8% of the diversity observed at the *COI* locus. Almost 90% of the variation in the *COI* locus was found within localities ($\Phi_{ST} = 0.103$, $P < 0.001$), while differences in genetic variation of localities within regions was marginally significant ($P = 0.054$). The Mantel test for isolation by distance showed a significant correlation between pairwise genetic distance and the log of geographic distance when all samples were included, $P = 0.044$ (Fig. 4). However, when only the California populations were considered (which are the locations of the most concentrated sampling effort) no correlation is observed between genetic and geographic distance, $P = 0.4189$.

Tajima's D and Fu and Li's D test were not significant within individual localities, but were negative and significant when the entire data set was considered (Table 1). This suggests that either selection or demographic factors could be influencing the genetic variation observed in the *COI* gene of *Profilicollis altmani* across its distribution. Fu's F_S statistic was negative within all populations, and in many populations these values were statistically significant ($P < 0.02$), indicating an excess of low-frequency haplotypes. The statistical significance of Fu's F_S across geographic localities suggests that this parasite may have experienced a period of rapid population growth in the past or that this locus is influenced by genetic hitchhiking (Fu, 1997). The mismatch distribution for *P. altmani* appears bimodal, indicating a difference between two clades of *COI* haplotypes; however, the distribution did not differ significantly from the unimodal model produced by ARLEQUIN ($P = 0.118$) (Fig. 5). In

Table 1 Molecular diversity indices and neutrality tests for *COI* sequences of *Profilicollis altmani* used in this study.

Locality	n	N_{hap}	h	π_1	π_2	Tajima's D	Fu's F_S	Fu & Li's D
CALIFORNIA								
Bodega	15	10	0.93 ± 0.05	4.17 ± 2.12	0.0069 ± 0.0041	−0.124	−2.782	−0.773
San Francisco	15	12	0.97 ± 0.03	4.02 ± 2.13	0.0067 ± 0.0039	−0.264	−5.925*	−0.449
Santa Cruz	15	13	0.97 ± 0.04	6.28 ± 2.70	0.0087 ± 0.0050	−0.396	−6.308*	−1.173
Moss Landing	15	11	0.97 ± 0.03	4.86 ± 2.51	0.0080 ± 0.0047	−0.052	−4.933*	−0.498
Monterey	18	14	0.97 ± 0.03	5.12 ± 2.60	0.0085 ± 0.0048	−0.286	−5.996*	−0.575
San Simeon	15	11	0.95 ± 0.04	4.78 ± 2.48	0.0079 ± 0.0046	−0.114	−3.511*	−0.498
Pismo Beach	21	12	0.94 ± 0.03	4.29 ± 2.21	0.0071 ± 0.0041	0.367	−3.092	−0.336
Santa Barbara	14	14	1.00 ± 0.03	5.28 ± 2.71	0.0087 ± 0.0050	−0.587	−10.318**	−1.098
Total	**128**	**42**	**0.94 ± 0.01**	**4.66 ± 2.30**	**0.0077 ± 0.0042**	**−1.267**	**−24.089****	**−4.062***
CHILE								
Antofagasta	15	11	0.95 ± 0.04	5.35 ± 2.73	0.0089 ± 0.0051	−0.190	−3.108	−0.537
Talcahuano	15	12	0.97 ± 0.03	4.75 ± 2.46	0.0079 ± 0.0046	−0.419	−5.114	−0.655
Total	**30**	**22**	**0.97 ± 0.01**	**4.96 ± 2.48**	**0.0082 ± 0.0046**	**−0.763**	**−13.356****	**−1.414**
ATLANTIC								
Rhode Island	11	9	0.96 ± 0.05	4.46 ± 2.38	0.0074 ± 0.0044	−0.350	−3.210	0.074
North Carolina	15	13	0.97 ± 0.04	6.27 ± 3.15	0.0104 ± 0.0059	−1.141	−5.383*	−1.528
Total	**26**	**17**	**0.96 ± 0.02**	**5.81 ± 2.87**	**0.0096 ± 0.0053**	**−0.872**	**−5.663***	**−1.094**
GULF								
Florida	15	8	0.90 ± 0.05	4.13 ± 2.18	0.0068 ± 0.0040	−0.690	−0.767	−1.074
Mississippi	4	1	0.00	–	–	–	–	–
Total	**19**	**8**	**0.81 ± 0.08**	**3.58 ± 1.90**	**0.0059 ± 0.0035**	**−0.822**	**−0.439**	**−1.320**
PANAMA	1	1	0.00	–	–	–	–	–
TOTAL	**204**	**72**	**0.96 ± 0.01**	**4.99 ± 2.44**	**0.0712 ± 0.0385**	**−1.786***	**−25.201****	**−5.797***

n, number of sequences; N_{hap}, number of haplotypes; h, haplotype diversity ± standard deviation; π_1, mean number of nucleotide differences ± standard deviation; π_2, nucleotide diversity ± standard deviation. For Tajima's D statistic and Fu and Li's D statistics, *indicates $P < 0.05$. For Fu's F_S, *indicates $P < 0.02$ and **indicates $P < 0.001$.

addition, the Harpending's raggedness index for the data was not significant ($P = 0.256$), indicating that the data were not ragged which would have been expected under a static population size, and thus we cannot rule out a past population expansion.

The isolation-with-migration model implemented in IMA estimated migration rates between the Pacific and Atlantic populations to be 34 migrants per generation to the Pacific and 24 to the Atlantic, although confidence intervals are large (90% highest posterior density confidence interval: 0.05–107.19; 0.02–81.96, respectively) despite agreement in values across four runs (mean number of migrants: 33.6–34.3 and 24.0–24.5, respectively) and an effective sample size of 60. These rates appear to have a high range considering the geographic distance. Migration between California and Chile was estimated to be even higher: from 82 migrants per generation into the California population to 729 migrants into Chile, again with large confidence intervals (0.05–333.94; 0.08–4876.62), despite agreement in estimates across runs and an effective sample size of 89, suggesting a limitation of the data. The time since divergence of the Pacific and Atlantic populations was estimated by the program to be between 28,500 and 71,400 years ago. Nested model analysis from IMA between populations in the Pacific and Atlantic showed that both models in which there was no migration could be rejected. Similarly, when only the populations from Chile and California were considered in a separate analysis all three models in

which migration was not included were rejected (Appendix S3). An additional model could be rejected if the theta for the population in Chile is the same as the ancestral, although the confidence intervals for these values suggest that this is an unlikely scenario (Appendix S3).

DISCUSSION

Lack of cryptic species diversity

Marine acanthocephalan parasites sampled from three species of *Emerita* mole crabs across wide geographic and temperature distributions show low genetic divergence. The low genetic variability among these acanthocephalans compared with that in previous studies in which cryptic acanthocephalan species were discovered in aquatic habitats is strong evidence that there are not cryptic species of acanthocephalans infecting the mole crabs *E. talpoida* and *E. analoga*. In addition, we observed several shared *COI* haplotypes between Pacific and Atlantic coastlines, thus there has not been wide genetic separation between ocean basins. This is supported by our isolation-with-migration analyses, which suggest significant gene flow between the Atlantic and Pacific, and especially between the coast of California and Chile. The high haplotype diversity observed at the *COI* locus, despite low nucleotide diversity and evidence of deviations from neutrality, also signals that there may have been a selective sweep
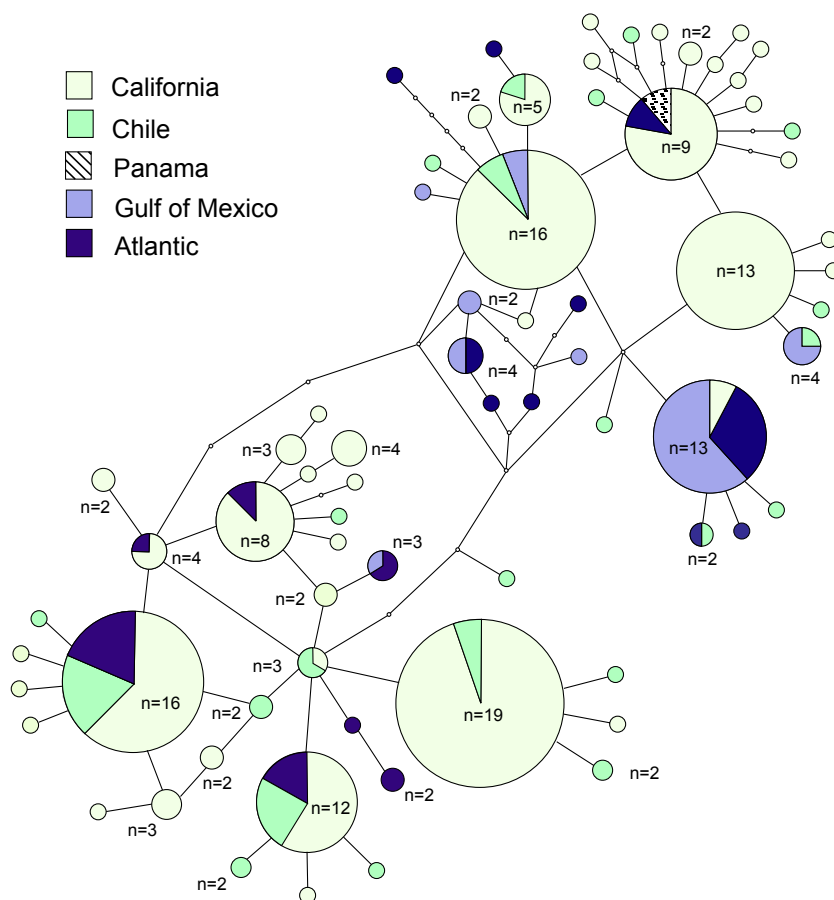
Figure 3 Parsimony haplotype network of *Profilicollis altmani*. Pie charts are scaled to represent the number of parasites sharing a particular *COI* haplotype; colors represent regional location (see key). Empty white circles indicate inferred mutational steps between haplotypes. Shared haplotypes range from 19 to 2 individuals (*n* = 19 to *n* = 2). Shaded haplotypes without labels are singletons.

Table 2 Pairwise comparison of genetic structure (Φ_{ST}) of *Profilicollis altmani* (below the diagonal) and F_{ST} values of genetic structure (above the diagonal). Bold numbers indicate values where $P < 0.05$.

	BO	SF	SC	ML	MO	SS	PI	SB	AN	TA	RH	NC	FL
BO		0.112	0.000	0.021	0.012	−0.028	0.031	0.047	0.636	0.065	**0.158**	0.014	0.056
SF	0.113		0.013	−0.027	−0.008	0.028	−0.028	−0.029	0.011	−0.020	−0.025	**0.133**	**0.282**
SC	0.000	0.012		−0.035	−0.032	−0.018	−0.030	−0.035	0.006	0.014	0.045	**0.058**	**0.146**
ML	0.021	−0.026	−0.036		−0.020	−0.022	−0.043	−0.052	−0.020	−0.031	0.009	**0.065**	**0.178**
MO	0.013	−0.008	−0.033	−0.020		−0.026	−0.031	−0.022	0.029	0.011	**0.040**	**0.066**	**0.160**
SS	−0.028	0.027	−0.019	−0.022	−0.026		−0.015	0.008	0.003	−0.009	0.053	0.016	**0.095**
PI	0.031	−0.028	−0.030	−0.043	−0.031	−0.016		−0.034	0.011	−0.017	0.021	**0.096**	**0.208**
SB	0.047	−0.029	−0.036	−0.052	−0.021	0.008	−0.035		−0.003	−0.001	0.010	**0.091**	**0.207**
AN	0.063	0.011	0.005	−0.020	0.029	0.003	0.011	−0.003		−0.009	−0.002	**0.060**	**0.182**
TA	0.066	−0.020	0.014	−0.032	0.011	−0.009	−0.017	−0.001	−0.009		0.009	**0.097**	**0.226**
RH	**0.160**	−0.025	0.045	0.010	0.040	0.053	0.021	0.011	−0.002	0.009		**0.103**	**0.261**
NC	0.013	**0.134**	0.058	0.064	0.067	0.015	**0.096**	**0.091**	0.060	**0.097**	**−0.104**		−0.012
FL	0.056	**0.284**	0.147	0.178	0.161	0.095	0.209	0.208	0.182	0.226	0.263	−0.013	0

Location abbreviations: BO, Bodega; SF, San Francisco; SC, Santa Cruz; ML, Moss Landing; MO, Monterey; SS, San Simeon; PI, Pismo Beach; SB, Santa Barbara; AN, Antofagasta; TA, Talcahuano; RH, Rhode Island; NC, North Carolina; FL, Florida.

that reduced genetic variation in this parasite or a recent population expansion across the oceans.

Our findings of a lack of cryptic species diversity were unexpected given prior studies on cryptic acanthocephalan diversity among freshwater hosts, which have revealed that some acanthocephalan species may only appear to be general-

ists until genetic diversity within the species is examined (Steinauer *et al.*, 2007; Martínez-Aquino *et al.*, 2009). Molecular analyses of freshwater acanthocephalans revealed substantial genetic variation among cryptic species ranging from 6.3% to 20% in *COI* and 1% to 11.7% in ITS, while genetic variation in *P. altmani* was only 1.8% in *COI* and there was
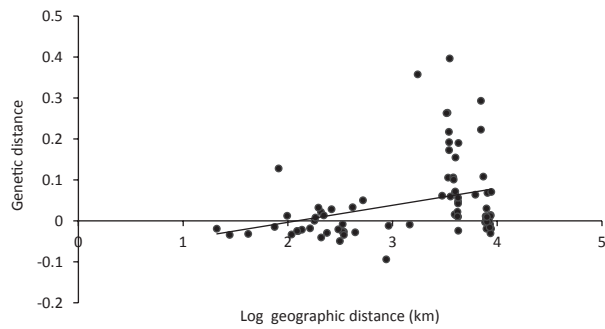
Figure 4 Isolation-by-distance test for *Profilicollis altmani*. There was an observed correlation between genetic distance ($\Phi_{ST}/1-\Phi_{ST}$) and log of geographic distance ($P = 0.444$) (samples from Mississippi and Panama were excluded due to low sample size).
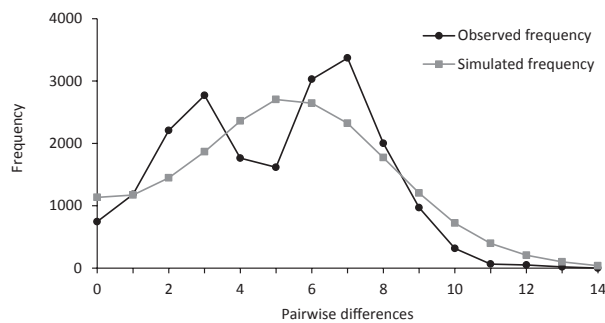

Figure 5 Mismatch distribution of pairwise distances among cytochrome *c* oxidase subunit I (*COI*) haplotypes in *Profilicollis altmani* compared with the expected frequency of pairwise differences (using Arlequin 3.1). Harpending's raggedness index was $r = 0.020$ and the corresponding *P*-value was 0.200.

no divergence in ITS1 or ITS2. The genetic diversity we observed is similar to the genetic diversity observed within a single cryptic lineage of freshwater acanthocephalan species. It is improbable that there are cryptic species of acanthocephalans undetected in *E. analoga* and *E. talpoida*; a meta-analysis of studies of parasite cryptic diversity found that the number of cryptic species discovered was most correlated with sequencing effort (Poulin, 2011). The sequencing of 204 parasites in this study is high among the sequencing effort analysed in Poulin's study. Thus, the lack of support for cryptic species of this acanthocephalan in this study is not likely to be an artefact of low sampling effort, and supports the idea that this parasite has maintained genetic connectivity between oceans or has recently colonized one ocean from the other.

Population structure and role of seabirds as vectors

Overall, populations of *P. altmani* showed little structure along the coast of California; Φ_{ST} values were low and not statistically significant, suggesting significant gene flow among these coastal sites or a recent range expansion. This level of gene flow is expected between nearby populations

due to the potential for dispersal of parasite eggs by movement of birds or possibly short distances by wave action and currents. Conversely, high genetic differentiation might be expected between populations in North and South America or between the Pacific and Atlantic coasts of North America. While the estimates are based on a single locus, these results suggest more gene flow between populations in the Northern and Southern Hemisphere of the Pacific than between the Atlantic and Pacific Ocean basins. However, pairwise distances and Φ_{ST} values showed high genetic similarity between most locations and IMA analyses suggest migration between distant populations, which together support the hypothesis of gene flow vectored by birds that transport the eggs large distances. Genetic homogeneity across a wide geographic distance has been observed in other free spawning marine invertebrates relying upon currents (Lessios *et al.*, 1998; Addison *et al.*, 2008). These examples suggest dispersal of acanthocephalan eggs across this range is feasible via transport of parasite propagules carried by host movements.

This acanthocephalan has been reported in a variety of birds which could disperse the parasite's eggs between populations, including *Melanitta* spp., *Larus* spp., *Marilla affnis*, *Crocethia alba*, *Calidris alpina*, *Numenius phaeopus hudsonicus* and *Catoptrophorus semipalmatus* (Karl, 1967). While many birds may not travel between the Pacific and Atlantic Oceans regularly, studies suggest that several bird species could be contributing to dispersal of this parasite between coastlines through seasonal migrations. The sanderling, *Crocethia alba*, migrates in an elliptical pattern between the Pacific and Atlantic coasts across North and South America (Myers *et al.*, 1990), while other birds such as the common tern, *Sterna hirundo*, and gull *Larus argentatus* have been found to migrate from eastern North America to the Carribean and may transverse central America to the Pacific coast (Rappole *et al.*, 2000). In addition, whimbrel, *Numenius phaeopus hudsonicus*, migrate seasonally between South America, the Caribbean and the Arctic (Watts *et al.*, 2008).

Once a parasite is brought to a new coast by a bird, the offspring of the parasite could be transported along the coast by other newly infected birds in a 'stepping stone' manner that would homogenize local genetic diversity. Along the coast of California, where sampling was most extensive, there was no relationship between pairwise genetic distance and the geographic distance between sites. A test of isolation by distance between all sampled populations showed a correlation between geographic distance and genetic distance; however, because there was not statistically significant genetic divergence between many geographically distant sites this is probably due to a relationship between *COI* haplotypes and the ocean basins. One hypothesis is that reduced migration across North and South America compared with coastal routes could result in lower dispersal and interchange between these distant populations, which is supported by our migration estimates from isolation-with-migration analyses. Additionally, the birds that migrate long distances may have a lower intensity of infection by this acanthocephalan

compared with the birds that are constrained to a smaller geographic area, and this could also contribute to reduced exchange between coasts.

In contrast to the genetic homogeneity between California and Chile, along the Atlantic coast of the USA population analyses showed there was genetic population differentiation between acanthocephalans in Rhode Island and those in Florida and North Carolina. Limited sampling along the east coast of North America constrains our ability to determine what may be causing the genetic differences between Rhode Island and the other Atlantic/Gulf acanthocephalan populations. While the land barrier between the Pacific and Atlantic is a probable explanation for the observed pattern of genetic diversity with the exception of Rhode Island, an alternative explanation would be that population structure in this acanthocephalan is influenced by environmental conditions, which might be observed either as a direct response to selection or as linkage with a mitochondrial locus. Water temperatures in North Carolina and the Gulf of Mexico are considerably warmer during the summer than average water temperatures in California, Chile and the northern Atlantic (NOAA, 2011) and would be a likely environmental variable to affect aquatic acanthocephalan eggs in the weeks they may endure before infecting a host (Kennedy, 1985, p. 387). This could result in populations from Rhode Island being more genetically similar to populations along the Pacific coasts of the USA and Chile than the geographically closer populations.

Signal of past demographic events or selection?

Neutrality tests showed evidence of deviation from neutrality for mutations in the *COI* locus in this acanthocephalan. While *COI* is often used in phylogenetic studies as a neutral marker, selection or genetic hitchhiking could act to reduce diversity of the locus and influence the distribution of haplotypes (Ballard & Kreitman, 1995; Barton, 2000), although this signal of selection may also be confounded by population expansion or other demographic events. Hitchhiking might be based on selection related to seasonal water temperature variation (as mentioned previously). Genetic distances are low between populations in Chile, California and Rhode Island that experience lower mean annual water temperatures, compared with populations in North Carolina and Florida with the highest summer water temperatures. The results of significant population structure despite gene flow and the results from the neutrality tests are also compatible with an explanation based on selection. The bimodal mismatch distribution of *P. altmani* is compatible with divergent selection towards two different haplotype clusters in this parasite, but the distribution could alternatively be evidence of past population expansions. Bayesian phylogenetic analysis of *COI* in the crab host *E. analoga* has suggested that the species may have undergone population expansions during periods of cooling of sea surface temperatures (Dawson *et al.*, 2011), and thus the parasite may have also undergone parallel changes in population size with their hosts.

CONCLUSIONS

This research shows that the acanthocephalan *P. altmani* has a broad distribution across North and South America and that cryptic species have not been found in *E. analoga* or *E. talpoida*. The lack of cryptic diversity in *P. altmani* was unexpected given the wide range of bird host species that this parasite has been reported in and the genetic and geographic isolation of the intermediate hosts between ocean basins, which provide opportunities for speciation. It is hypothesized that the great diversity of marine parasites has been founded by generalist parasite species which break off into new lineages of specialist species; however, parasite species may be able to maintain a generalist strategy when costs in losing definitive hosts in which they can complete their life cycle are high (Palm & Klimpel, 2006). In this case, it could be that the variety of birds that consume mole crabs leads to unpredictability regarding the final host, and therefore limits parasite host specialization.

A lack of population structure has also been found among other types of seabird parasites and shows that mobility of birds has the potential to homogenize genetic diversity among parasite populations across a broad range (McCoy *et al.*, 2003; Stefka *et al.*, 2009). Recent studies have suggested that multiple *Profilicollis* species in North America are synonyms with *P. altmani* (Nickol *et al.*, 2002). The genetic results of this study go further and suggest that the acanthocephalans infecting the mole crabs in the USA and Chile are also the same species, first described as *P. altmani*. The geographic range of this acanthocephalan may also spread into the tropics; acanthocephalans have been observed in the mole crabs in Panama (M. Torchin, Smithsonian Tropical Research Institute, pers. comm.) and the one found in a single Panamanian crab sample in this study had identical *COI* and ITS haplotypes as individuals from California and the Atlantic coast.

This first biogeographic examination of a marine acanthocephalan shows they may have broad geographic distributions in which they parasitize multiple species of intermediate hosts and diverse species of definitive hosts. We infer that the acanthocephalan parasitizing mole crabs around North and South America is most likely a single species with broad thermal tolerance. While the crabs *E. analoga* and *E. talpoida* were sampled from multiple locations across their range, additional sampling is needed of acanthocephalans from the other *Emerita* species. This parasite appears to be a generalist parasite that utilizes a variety of marine birds as final hosts, and differs from previously studied acanthocephalans that parasitize fish in freshwater and brackish environments, which were shown to be multiple specialized lineages. Sequencing of additional genes in this acanthocephalan in the future could determine whether a population expansion or genetic hitchhiking is a more likely explanation for the patterns of genetic diversity observed. It would also be intriguing to compare patterns of cryptic diversity and population structure of other marine acanthocephalan

species that parasitize migrating hosts to examine how the life history of these hosts influences genetic diversity and speciation of these parasites.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Crab sampling data.
Appendix S2 Occurrence of *COI* haplotype by locality.
Appendix S3 Results of the isolation-with-migration analysis.

BIOSKETCHES

Tricia Goulding is a PhD student at the Pennsylvania State University. This work is based on her Master's thesis at the Romberg Tiburon Center, San Francisco State University. T.G. is interested in biodiversity and population connectivity, especially in marine species. Her research currently focuses on the systematics, taxonomy and phylogeography of the onchidiid slugs across the Indo-West Pacific.

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